SHORT REPORT

Mitochondrial Aspartate/Glutamate Carrier SLC25A12 Gene Is Associated With Autism

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Two single nucleotide polymorphisms (SNP) within Mitochondrial Aspartate/Glutamate Carrier *SLC25A12* gene have recently shown to be strongly associated with autism. Here, we attempted to replicate this finding in two separate Finnish samples with autism spectrum disorders. Family-based association analysis of two SNPs, rs2056202 and rs2292813, previously shown to be associated with autism was performed in two samples with different phenotypic characteristics. The samples included 97 families with strictly defined autism and 29 extended families with Asperger syndrome (AS). We detected association at rs2292813 (FBAT, $P = 0.0018$) in the Finnish autism sample. In, addition other family-based analysis methods supported this finding. By contrast, analysis of the AS sample yielded no evidence for association. This study shows further support that genetic variants within *SLC25A12* gene contribute to the etiology of autism.

Keywords: autism; Asperger syndrome; SLC25A12; association; SNP

Introduction

Autism (MIM 209850) is a severe neurodevelopmental disorder characterized by abnormalities in reciprocal social interaction and communication, restricted and stereotyped patterns of interests and activities, and the presence of developmental abnormalities, which are evident by the age of 3 years [Ameican Psychiatric Association, 1994; World Health Organization, 1993]. More broadly defined, the group of autism spectrum disorders (ASDs) includes also Asperger syndrome (AS) and atypical forms of autism. Based on twin, family, and molecular genetic studies, autism seems to have a strong but complex genetic background [Veenstra-VanderWeele & Cook, 2004]. There is now evidence that in some cases rare strong effect mutations may lead to autistic phenotype. An alternative possibility is that a combination of small effect susceptibility variants potentially combined with effects of environmental factors contribute to the phenotype [Bailey et al., 1995].

In genetic association studies, replication of the initial findings in independent study samples is an essential step in establishing confirmed susceptibility variants. This has turned out to be an extremely difficult task especially in the neuropsychiatric diseases [Glatt & Freimer, 2002]. However, a few promising associations have recently been reported in autism, one of which is for Mitochondrial Aspartate/Glutamate Carrier *SLC25A12* (AGC1). *SLC25A12* gene is located at 2q31.1 originally implicated by several independent linkage studies. A study by Ramoz and colleagues reported mutation analysis of nine candidate genes across 2q31 region and two of the identified genetic variants located within *SLC25A12* were strongly associated with autism [Ramoz et al., 2004]. Additional support for this finding was recently reported in an independent study sample of 167 autism trios [Segurado et al., 2005] as well as the findings showing that these variants might contribute to the variability of routines and rituals associated with autism [Silverman et al., 2007]. However, also findings of no association have been reported [Blasi et al., 2006; Rabionet et al., 2006].

In this study, we attempted to replicate the reported association between *SLC25A12* gene and autism in the Finnish families and hypothesized that in the case of true association it has to be present in different study samples sharing similar population history and it has to be detectable by analysis of the same variant(s) across the study samples.

Samples and Methods

Two different samples of Finnish families with ASDs were included in the analyses. Autism sample (Sample 1)

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included a total of 97 families with at least one affected individual with strictly defined autism based on *Diagnostic and Statistical Manual of Mental Disorders*—IV [DSM-IV; Ameican Psychiatric Association, 1994] and *International Classification of Diseases* —10 [ICD-10; World Health Organization, 1993] criteria. A total of 66 families were trios and 31 families had multiple children affected with autism [Auranen et al., 2002]. Asperger sample (Sample 2) comprised 29 extended families, which included up to ten individuals diagnosed with AS based on ICD-10 criteria [Rehnstrom et al., 2006; Ylisaukko-oja et al., 2004]. The total number of affected individuals was 118 and 114 in the autism and Asperger samples, respectively. The research was approved by the Ministry of Social Affairs and Health (Finland), the appropriate institutional review boards, and the local ethical committee, with written informed consent obtained from all individuals involved in the study.

We genotyped two SNPs, rs2056202 and rs2292813 located within *SLC25A12* gene by using fluorogenic 5́ nuclease allelic discrimination chemistry (TaqMan®, Applied Biosystems, Foster City, CA) with an ABI Prism® 7900 Sequence Detection System (Applied Biosystems, Foster City, CA). The assay mix containing primers and probes was designed by Applied Biosystems. Genotype data were tested for Mendelian errors and none was detected. All duplicated samples were coherent and the markers were in Hardy–Weinberg equilibrium. The overall genotyping success rate exceeded 99%.

We used three different family-based association analysis methods. First, we employed traditional Haplotype Relative Risk (HRR 2xn) analysis as implemented in the Analyze package [Hiekkalinna et al., 2005]. Second, we performed FBAT analysis (http://www.biostat.harvard.edu/~fbat/fbat.htm) for both single markers and two-marker haplotype with empirical variance option, as appropriate in the presence of linkage and when data of multiple sibs in a family are available. Third, Pseudomarker analysis with linkage disequilibrium (LD) given linkage option was performed. This method is capable of incorporating various types of data in the analysis as well as analyzing linkage and association both separately and jointly [Goring & Terwilliger, 2000]. Pseudomarker should be well suited especially for the analysis of extended AS families (Sample 2). The extent of LD between single nucleotide polymorphisms (SNPs) was estimated by HaploView-program [Barrett et al., 2005]. Only the individuals fulfilling strict diagnostic criteria for autism (Sample 1) or AS (Sample 2) were assigned as affected in the analyses.

Results

The two SNPs are located 68.2 kbs apart and the extent of LD between SNPs ($D' = 1.0$; $r^2 = 0.51$) in the total Finnish sample was comparable to the extent of LD in the Caucasian (CEPH) sample of the HapMap data (www.hapmap.org). Based on Caucasian HapMap data, the SNPs are located within a large haplotype block extending 389 kb. In the autism sample (Sample 1), we detected association at rs2292813 by all the three analysis methods used, the best *P*-value being 0.0018 using the FBAT. The common allele (G) was overtransmitted in accordance with the original report [Ramoz et al., 2004]. By contrast, analysis of rs2056202 yielded no evidence of association (Table I). The G allele at rs2292813 was transmitted to the patients 179 times, and nontransmitted 133 times (HRRx2). The A allele was transmitted to the patients 11 times, and nontransmitted 23 times (HRRx2). The Haplotype analysis of SNPs rs2292813- rs2056202 by FBAT yielded in global *P*-value of 0.003, the best individual haplotype being A-A with *P*-value of 0.0018. Other haplotypes yielded in $P = 0.224$ (G-G) and $P = 0.147$ (G-A). The frequencies of the haplotypes were 0.854 for G-G, 0.054 for G-A, and 0.092 for A-A, respectively. The haplotype A-A was undertransmitted to the patients (observed 12 times, expected 20.8 times). In the Asperger sample (Sample 2), all the analyses for individual markers or for two-marker haplotype resulted in $P > 0.05$.

Discussion

We set out to test association between the *SLC25A12* gene and autism and hypothesized that in the case of true finding association should be detectable by analyzing the same SNPs that showed positive evidence in the original study. We detected evidence for association at rs2292813

Table I. Association Results of *SLC25A12* Variants in the Finnish Autism and Asperger Syndrome Samples

			Autism (Sample 1)			Asperger (Sample 2)		
SNP	Location[a]	MAF[b]	HRR 2xn	FBAT	Pseudomarker	HRR 2xn	FBAT	Pseudomarker
rs2292813	172469736	0.09	**0.0054**	**0.0018**	**0.0074**	0.3266	0.4913	0.0531
rs2056202	172537987	0.15	0.1911	0.2237	0.2191	0.9824	NA	0.8231

[a]UCSC Human Genome Browser, May 2004 assembly.
[b]Minor allele frequency in current data set.

as well as for the two-marker haplotype in the Finnish sample of autism families.

These findings are encouraging in several respects. First, the original finding was based on a systematic analysis of positional candidates within a well-replicated linkage region for autism [Ramoz et al., 2004]. Furthermore, association for the SNPs analyzed here have been also replicated in an independent sample [Segurado et al., 2005]. Thus, it is clear that the variants analyzed in this study have strong prior probability, which inevitably decreases the risk of false-positive results. In addition, the fact that association was detected in a sample of limited size might indicate relatively strong contribution to the etiology. It is also encouraging that similar evidence for association was detected by all the analysis methods tested, decreasing the possibility of method-dependent false-positive finding. Importantly, the overtransmitted allele of rs2292813 in this study was consistent with the earlier positive reports [Ramoz et al., 2004; Sequardo et al., 2005]. However, the haplotype (A-A) showing best association was different from the earlier studies (G-G).

Despite promising findings at the *SLC25A12* gene it is important to note that also negative reports exist [Blasi et al., 2006; Rabionet et al., 2006]. Therefore, it is important to perform combined data analyses or to employ meta-analysis methodology to further determine the significance of *SLC25A12* variants across different samples as well as to analyze yet additional autism samples to further elucidate potential role of *SLC25A12* in the etiology of autism.

Independent replication studies have a critical role in confirming or disproving the reported genetic associations. Although some promising associations have recently emerged for autism, inability to replicate original findings has still been the predominant phenomenon. Reported data are also often difficult to interpret owing to inconsistency of variants that have been analyzed across the studies, association findings for different genetic variants in different studies, and modest significance levels of the reported associations. Furthermore, candidate gene studies commonly consider hypothesis-driven candidates, which have been selected because of their role in potentially relevant pathways for the disease. In such cases, prior probability is usually extremely low and highly convincing association evidence would be required to reject the null hypothesis.

Currently, there are also important functional data available, which shows that Mitochondrial Aspartate/Glutamate Carrier encoded by *SLC25A12* is more strongly expressed in postmortem brain tissues of autistic subjects compared with those of controls in specific brain regions. It was hypothesized that this may lead to modifications of neuronal networks in specific subregions, such as dorsolateral prefrontal cortex and fusiform gyrus, at both prenatal and postnatal stages of development, thereby contributing to the pathophysiology of autism [Lepagnol-Bestel et al., 2008].

Taken together, SLC25A12 remains one of the most promising candidates for autism and also this study supports the hypothesis that *SLC25A12* is involved in conferring risk for autism. However, extensive studies are needed to confirm the role of *SLC25A12* in the pathogenesis of autism as well as to identify the actual predisposing allelic variant(s) from this genomic region.

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